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SHORT COMMUNICATION

Epidemiology and detection of acinetobacter using conventional culture and in-house developed PCR based methods

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Summary Active surveillance cultures for multidrug-resistant (MDR) gram-negative bacteria is one strategy to control outbreaks. The objectives of the study are to evaluate the prevalence of *Acinetobacter* colonization and to compare conventional culture and in-house developed PCR based method. Swabs were collected from patients transferred from another organization or were admitted to the intensive care units. Swabs were cultured by conventional method and were tested using in-house LightCycler® 2.0 real-time PCR method. Of 449 tested samples, the majority came from cardiac step down unit (188, 42%), male medical floor (80; 18%), and coronary care unit (66; 13.4%). Of the total specimens, 14 (3%) were positive by PCR and 12 (2.6%) were positive by routine cultures. The positivity rates among wounds, respiratory, perineal, and nasal samples were 3.2%, 9.7%, 4.6% and 0.8% respectively. Two positive samples by PCR were negative by routine culture.

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The overall concordance rate was 99.5% and the positive concordance rate was 85.7%. The current study revealed a low prevalence of MDR *Acinetobacter* among the studied population. The LightCycler® 2.0 PCR produced comparable positive results to routine cultures.

Introduction

Acinetobacter spp. in general and *Acinetobacter baumannii* in particular are increasingly recognized pathogens in the healthcare setting leading to healthcare associated infections [1—3]. The emergence of multi-drug resistant (MDR) *Acinetobacter* is of particular importance and necessitates the development of rapid and sensitive molecular detection methods to institute the appropriate therapeutic and infection control measures. Such methodology may rely on PCR or loop-mediated isothermal amplification (LAMP) assay [4—6]. In Saudi Arabia, many studies showed high percentage of carbapenem resistant *A. baumannii* [7—9]. Active surveillance cultures for multidrug-resistant (MDR) gram-negative bacteria is one strategy to control outbreaks among patients in intensive care units and patients transferred from facilities with high prevalence rates of MDR organisms [10,11]. The effectiveness of screening cultures for the detection of MDR *Acinetobacter* was 55% even if six samples were obtained [12].

We preemptively developed a screening program to screen patients admitted to the intensive care unit and those admitted from other hospitals. Those patients were put on contact isolations and screened for *Acinetobacter*. Since, routine cultures take longtime for the results; we sought to develop a rapid method for the detection of *Acinetobacter* spp. and to evaluate the prevalence of *Acinetobacter* among this cohort of patients.

Materials and methods

This is a prospective study in a general hospital with 350 beds. Swabs were obtained from the nose and wounds. A total of 449 different multi-site swabs were obtained between January and July 2014.

Laboratory processing and culture methods

The swabs were inoculated on Blood agar plate and CHROMACIN *Acinetobacter* selective agar (Saudi Prepared Media laboratory SPML). Identification and antibiotic resistance testing were done after 24 h of incubation at 37 °C and 5% CO_2 using Vitek2 system (BioMérieux). MDR was defined as resistance to more than two of the following classes: antipseudomonal cephalosporins, antipseudomonal carbapenems, ampicillin/sulbactam, fluoroquinolones, and aminoglycosides [13].

DNA extraction

DNA was extracted using the MagNA-Pure-Compact-System (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. The swabs were broken into 1.5 Eppendorf tube, and 1 ml of sterile 0.9% NaCl was added and the mixture was vortexed vigorously. A total of 400 μl of the sample suspension was pipetted into MagNa pure sample tube and DNA extraction was performed using DNA-bacteria protocol. The DNA sample was eluted in 50 μl total volume.

Primer design

The primers and probes targeting 16S RNA sequence of *Acinetobacter* spp. were designed by TIB Molbiol (Syntheselabor GmbH, Eresburgstr, Berlin). The forward primer was Acineto_S: 5-ACAgCgATg-TgATgCTAA-3 and the reverse primer was: Acineto_B: 5-TATTCACCgCggCATT-3. To increase the specificity of the PCR, two internal oligonucleotide hybridization probes: Acineto_FLU probe: 5-TTgCAgACTCCAATCCgg—FL (18 bp, Tm = 56.3) and LCRED640-CTACgATCggCTTTTTgAgAT—PH (21 bp, Tm = 54.5) were added. The size of the amplicon was 115 bp.

PCR amplification

PCR amplifications were carried out in 10-μl volumes containing 2.5 μl of template DNA as shown in Table 1. The PCR conditions of the assay was as follows: after 10 min at 95 °C for FastStart Taq DNA polymerase activation, 30 amplification cycles were performed, each with 5 s denaturation at 95 °C,

Table 1 Results of routine cultures and PCR testing.

	Acinetobacter	MDR Acinetobacter	Negative culture	Total
PCR positive	3	9	2	14
PCR negative	0	0	435	435
Total	3	9	437	449

Fisher's exact test: two-tailed *P* value equals 0.8427.

5 s annealing at 60 °C and 15 s elongation at 72 °C on a LightCycler® 2.0 instrument. After amplification, a melting curve analysis was completed after 20 s denaturation at 95 °C. Samples were incubated at 56 °C for 20 s by continuous heating to 85 °C with a slope of 0.2 °C/s. Samples from each strain were diluted, from isolated colony to approximately 0.5 McFarland. A total of 400 μl of the suspension was used to extract the DNA. Acinetobacter ATCC#BAA747 was diluted to approximately 0.5 McFarland. The suspension was diluted to reach approximately 100—150 CFU/swab. Each dilution was run in replicates of four and adsorbed onto Copan swab (Copan 139C; Copan Italia SPA, Brescia, Italy).

Results

Analytical specificity of the real time PCR assay

The primers and probes were specific for 16S RNA sequence *Acinetobacter spp*. A total of 72 ATCC strains were used to evaluate the cross-reactivity. These included *Streptococcus pneumoniae* ATCC 49619, *Klebsiella pneumoniae* ATCC 700603, *Streptococcus agalactiae* ATCC 12386, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus epidermidis* ATCC 12228, *Haemophilus influenzeae* ATCC 1011, *Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29217, and Acinetobacter ATCC#BAA747. All bacterial strains and human DNA were tested and revealed no signal amplification, indicating that the assay is specific.

Melting curve analysis

Melting curve analysis of *Acinetobacter spp*. amplified PCR products revealed a single distinct melting peak at a mean of 61.52 °C (±2SD) for all tested ATCC and culture positive strains. The expected size of the PCR product was confirmed by gel electrophoresis and determined as 115 bp.

Lower limit of detection of the real time PCR assay

The lower dilution (150 CFU/swab) showing positive results in all four replicates was considered the analytical sensitivity.

Epidemiology of *Acinetobacter*

The majority of the samples came from cardiac step down unit (188, 42%), male medical floor (80; 18%), and coronary care unit (66; 13.4%). The majority of patients were male (61.7%). Of all the samples, 106 (23.6%) were perineal swabs and 122 (27.2%) were nasal swabs.

Comparative study results

Of the total specimens, 14 (3%; 95% CI: 1.2—4.1) were positive by PCR and 12 (2.6%; 95% CI: 1.5—4.7) were positive by routine cultures (*P* value = 0.84). The positivity rates among wounds, respiratory, perineal, and nasal samples were 3.2%, 9.7%, 4.6% and 0.8% respectively. There were 2 positive samples by PCR and were negative by routine culture (Table 1). The overall concordance rate was 99.5% and the positive concordance rate was 85.7% (Table 2). The two PCR positive culture negative samples were sub-cultured on BA and screened again for the presence of *Acinetobacter*. Both samples grew *Acinetobacter* colonies.

Table 2 Concordance Rate between the results of routine cultures and PCR testing.

		Routine culture		
		Positive	Negative	Total
PCR	Positive	12	2	14
	Negative	0	435	435
	Total	12	437	449

Positive concordance rate 12/14 = 85.7%.
Overall concordance rate 437/449 = 97.3%.

Antibiotic susceptibility

Of the 12 culture positive samples, 9 (75%) had MDR *Acinetobacter* and this represents 2% of all tested samples.

Discussion

In this study, we compared the performance of an in-house LightCycler® 2.0 assay (Roche Applied Science) and a standard culture method for detection of *Acinetobacter* from 452 swabs. The overall positive rate for *Acinetobacter* was about 3% of all tested samples. Another study from two Saudi Arabian hospitals utilized 565 rectal swabs and 8.3% showed *Acinetobacter* spp. [14]. In the current study, 2% of the total samples were MDR *Acinetobacter* and represented 75% of the positive cultures. In the same institute, MDR *Acinetobacter* was observed in 14—35.8% previously [15]. In a study of 17,760 fecal specimens, 1.9% specimens were MDR *Acinetobacter* [16] and 6.2% in another study [14]. This is in contrast to a recent study from a nursing home where 25 of 168 (15%) had MDR *A. baumannii* [17].

The best methods for active screening and the best specimen to detect colonization with *A. baumannii* are not known [18]. In the current study, a higher percentage of positivity for *Acinetobacter* was among throat samples (9.7%). In a study of carbapenem-resistant *A. baumannii* screening among known positive patients, the most frequent sites of isolation of this organism were tracheal aspirates (80%), rectal specimens (69%), sternal skin swabs (52%), and urine (25%) [19]. In another study, prevalence of *Acinetobacter* was 4.9%, 6.5% and 4.2% from rectal swabs, pharyngeal swabs, and tracheal aspirates, in patients on mechanical ventilation [20].

Active screening for the detection of *A. baumannii* was mathematically calculated to be cost saving (19—53% reduction in mean hospital cost per patient) except at a carrier prevalence of $\leq$2% and screening test sensitivity of $\leq$55% [21]. Given the overall prevalence of *Acinetobacter* spp in the current study of 3%, active screening is cost effective only for those patients who are at high risk of acquisition of *Acinetobacter* in our population.

The LightCycler® 2.0 PCR produced comparable positive results (3%) to routine cultures (2.6%). The failure of the culture to identify two samples likely related to the overgrowth of other microorganisms. Thus, the PCR methodology could be used to screen patients for *Acinetobacter* and allow fast turnaround time of 2 h compared to 3—5 days by standard culture method. This has an impact on infection control and isolation of infected or colonized patients. However, a positive PCR in this study could not differentiate between MDR and susceptible *Acinetobacter*. Thus, MDR *Acinetobacter* PCR detection is desirable when the prevalence of *Acinetobacter* is high.

The limitation of the current study includes: inability to differentiate different species of *Acinetobacter* and inability to detect MDR *Acinetobacter*. In areas with low prevalence of *Acinetobacter* in general, the use of the current PCR methodology would provide rapid identification of colonized patients and those then could be screened using conventional method.

Funding

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Competing interests

None declared.

Ethical approval

The Study was approved by the Johns Hopkins Aramco Healthcare Institutional Board.

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